Journal of Current Medical Research and Opinion

**CMRO 09 (08) 4827-4844 (2026)**
*https://cmro.in/index.php/jcmro/article/view/1205/1131*
**www.cmro.in**

CMRO
ISSN (O) 2589-8779
(P) 2589-8760

*Original Research*

# Analyzing the Bioactive Natural Compounds Produced by Klebsiella pneumoniae and Their Antimicrobial Secondary Metabolites via Gas Chromatography-Mass Spectrometry

Dr. Imad Hadi **Hameed**

Department of Medical Biotechnology, College of Biotechnology, AL-Qasim Green University, Iraq

**Abstract**

Secondary metabolites produced by microbes have low molecular mass and have peculiar structural features. Various biological functions, such as antibacterial agents, are displayed by the structurally varied metabolites. Klebsiella pneumoniae methanolic extract contained 39 bioactive chemicals. GC-MS analysis of Klebsiella pneumoniae revealed the existence of the Tricyclo[4.3.1.1(3.8)]undecan-1-amine, 3-Methoxybenzaldehyde semicarbazone, carboxaldehyde , 1-methyl-,oxime ,(Z)-(+), 1,5,5-Trimethyl-6-methylene-cyclohexene, 4-(2,5-Dihydro-3-methoxyphenyl)butylamine, Paromomycin , 9-Borabicyclo[3.31]nonane , 9-mercapto-, Benzenemethanol , 2-(2-aminopropoxy)-3-methyl, Acetamide , N-(6-acetylaminobenzothiazol-2-yl)-2-(adamantan, rin-6-carboxylic acid , 4-(2,5-Dihydro-3-methoxyphenyl)butylamine, N-(2,5-Dicyano-3,4-dihydro-2H-pyrrol-2-yl)-acetamide, 3,10-Dioxatricyclo [4.3.1.0(2,4)]dec-7-ene, 3-Cyclohex-3-enyl-propionic acid, Eicosanoic acid ,phenylmethyl ester, 3,7-Diazabicyclo[3.3.1]nonane , 9,9-dimethyl-, Dithiocarbamate , S-methyl-,N-(2-methyl-3-oxobutyl)- , dl-Homocysteine, 2-(2-Furyl-4-O-difluoromethyluracil, Uric acid, Pyrrolo[1.2-a]pyrazine-1,4-dione , hexahydro-,12-Methyl-oxa-cyclododecan-2-one, Phthalic acid , butyl undecyl ester, 9,12,15-Octadecatrienoic acid , 2,3-bis(acetyloxy)propyl ester, 1,2,4-Trioxolane-2-octanoic acid 5-octyl-, methyl ester, 12-Dimethylamino-10-oxododecanoic acid , Octahydrochromen-2-one, L-Aspartic acid , N-glycyl-,2H-Oxecin-2-one , 3,4,7,8,91,10-hexahydro-4-hydroxy-10-meth , Thiazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione , 2-amino-4-(2-ph, Dec-9-en-6-oxo-1-ylamide, 3,6,12-Trimethyl-1,4,7,10,13,16-hexaaza-cyclooctadecane, 2-Iodohiistidine, 2,5-Piperazinedione ,3,6-bis(2-methylpropyl)-, 9-Octadecenamide , (Z)-, 3',8,8'-Trimethoxy-3-piperidyl-2,2'-

Corresponding Author: ***Dr. Imad Hadi Hameed***, Department of Medical Biotechnology, College of Biotechnology, AL-Qasim Green University, Iraq.
Email Id: imadbiotechnology@gmail.com


**Received:** 01.07.2026 | **Revised:** 15.08.2026 | **Accepted:** 19.07.2026 | **Published:** 31-08.2026

binaphthalene-1,1',4,4'-tetra. Clinical pathogens selected for antibacterial activity namely, *Streptococcus pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Staphylococcus epidermidis*, It were 4.09±0.013, 2.99±0.300, 4.37±0.200, 3.22±0.210, and 4.00±0.203 respectively for Bacterial products (Metabolites Produced by *Klebsiella pneumoniae*), while recorded 1.08±0.200, 0.97±0.116, 2.08±0.233, 3.04±0.261, 0.98±0.166 respectively for Bacterial products Streptomycin antibiotics, and recorded 1.02±0.180, 1.00±0.190, 2.08±0.236, 1.00±0.100, and 1.82±0.200 respectively for Kanamycin antibiotics. *Klebsiella pneumoniae* produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *Klebsiella pneumoniae* can be useful.

**Keywords:** Bioactive Natural Compounds, Klebsiella pneumoniae, Antimicrobial, Metabolites

**Introduction:**

A rod-shaped, facultative anaerobic, Gram-negative, nonmotile bacteria, Klebsiella pneumoniae ferments lactose. According to references [1-3], it grows on MacConkey agar as a mucoid lactose fermentor. Klebsiella bacteria usually display two kinds of cell surface antigens. There are nine distinct types of lipopolysaccharide (LPS), one of which is O antigen [4]. Second, there are about eighty different kinds of K antigen, a capsular polysaccharide [5-7]. Both are essential for serogrouping and play a role in pathogenicity. Because of its nitrogen-fixation mechanism and the fact that K. pneumoniae has been shown to boost crop yields in agricultural settings, this free-living diazotroph has attracted a lot of attention from scientists and is of interest to farmers [8]. Outside of healthcare settings, bronchopneumonia and bronchitis caused by Klebsiella bacteria account for the vast majority of cases of pneumonia. Lung abscesses, cavitations, empyemas, and pleural adhesions are more common in these individuals. Even when treated with antibiotics, the mortality rate is close to 50%. People who are alcoholics and have bacteremia have a death rate that is close to 100% .[9]

This bacteria is normally present in the skin, intestines, and mouth, but it can cause serious damage to the lungs of humans and animals if they breathe it in. It targets the alveoli in the lungs, which can lead to bloody sputum. It is the most important Klebsiella bacterium in the clinical context, belonging to the Enterobacteriaceae family. Human clinical specimens have also shown the presence of K. oxytoca and K. rhinoscleromatis. Nosocomial infections caused by Klebsiella species have emerged as a major health concern in the past few years. Klebsiella is a potential cause of pneumonia as well as infections in the lower biliary system, surgical incision sites, and the urinary tract [10]. A variety of clinical conditions can manifest, including but not limited to: pneumonia, thrombophlebitis, UTI, cholecystitis, diarrhoea, URTI, wound infection, osteomyelitis, meningitis, bacteremia, and septicaemia. There is a higher risk of infection for patients who have an intrusive device in their body, such as those with respiratory support equipment, urine catheters, or devices used in neonatal wards [11–14]. Another risk factor for nosocomial infection with Klebsiella bacteria is the use of antibiotics. Bacterial infection in the bloodstream can cause sepsis and septic shock.

**The objectives of this study were :**

1. Analysis of secondary metabolites produced by *Klebsiella pneumoniae* Gas Chromatography-Mass Spectrometry (GC-MS).
2. Evaluation antimicrobial activity of secondary metabolites produced by *Klebsiella pneumoniae*

**Enterobacteriaceae**

**Enterobacteriaceae** is a large family of Gram-negative bacteria. It was first proposed by Rahn in 1936, and now includes over 30 genera and more than 100 species. Its classification above the level

of family is still a subject of debate, but one classification places it in the order Enterobacterales of the class Gammaproteobacteria in the phylum Pseudomonadota.

Enterobacteriaceae includes, along with many harmless symbionts, many of the more familiar pathogens, such as *Salmonella*, *Escherichia coli*, *Klebsiella*, and *Shigella*. Other disease-causing bacteria in this family include *Enterobacter* and *Citrobacter*. Members of the Enterobacteriaceae can be trivially referred to as enterobacteria or "enteric bacteria", .

**Pathogenic bacteria**

Germs that are capable of causing illness are known as pathogenic bacteria. Bacteria that cause illness in people are the main topic of this piece. Some bacteria can cause infectious diseases, while the vast majority of bacterial species are benign and even helpful. It is believed that there are less than 100 of these harmful species in the human population. The gut flora, on the other hand, consists of thousands of species [12]. Many different kinds of bacteria are always present in the human body. Some of these bacteria are helpful commensals that live on the skin and in the mucous membranes. Other bacteria are saprophytes, and they mostly live in soil and decaying materials. The nutrients found in bodily fluids, such as blood and tissue fluids, are enough to support the growth of numerous bacteria. The immune system and other defence mechanisms of the body allow it to withstand the invasion of many different types of microbes and keep them from damaging its tissues. Invading areas of the body, like the bloodstream, where bacteria are not typically present, is possible because pathogenic bacteria are uniquely suited and equipped to overcome the normal defences of the body. A large number of pathogens penetrate deeper layers of epithelium, skin, or mucous membranes, before dispersing via lymphatic and blood streams. An infectious germ can infect a healthy person in extremely rare instances; however, infection typically happens when the body's defensive mechanisms are compromised due to local trauma or a debilitating condition, such as wounds, intoxication, chills, exhaustion, or malnutrition. Distinguishing between an infection and colonisation, in which the bacteria are not actively harmful, is crucial in many situations [13]. Bacteria that cause pneumonia (Staphylococcus, Streptococcus, and Pseudomonas) and food poisoning (Shigella, Campylobacter, and Salmonella) are pathogenic and contribute to other serious diseases that affect people all over the world. Diseases like leprosy, syphilis, diphtheria, tetanus, and typhoid fever can be caused by pathogenic microorganisms as well [14]. Infant mortality rates in underdeveloped nations are largely attributable to pathogenic microorganisms. One in eight deaths, or approximately 7.7 million deaths, in 2019 could have been caused by infections, according to a GBD study that evaluated the global death rates from 33 different bacterial pathogens [16,17]. Cultures of most harmful bacteria can be recognised using techniques like Gramme stain. It is common practice to test antibiotic efficacy on bacteria cultured in this manner. To prove a causal association between a bacterium and a disease, Koch's postulates are used as a standard for hitherto unknown pathogens.

***Klebsiella***

A genus of rod-shaped, Gram-negative, oxidase-negative bacteria known as Klebsiella is characterised by a capsule made of polysaccharides. [18] Species of Klebsiella are ubiquitous in the natural world. This is believed to be because different sublineages have adapted to different niches, with related metabolic changes that make them more suitable to a certain setting. You can find them in all sorts of places, including water, dirt, plants, insects, and even people [19,20]. The germ klebsiella was named after the German-Swiss scientist Edwin Klebs (1834–1913). For a long time, Klebsiella bacillus was known as Friedlander bacillus because of Carl Friedlander's description of it. Klebsiella bacteria are common in the nasal passages, oral cavity, and digestive tracts of both humans and other animals. All Klebsiella species are gram-negative and

typically do not have motile cells. In comparison to other members of the Enterobacteriaceae family, they are typically shorter and thicker. The typical dimensions of the rod-shaped cells are 0.3 to 1.5 µm in width and 0.5 to 5.0 µm in length. They often appear alone, in pairs, or even in long strings. Similar to other members of the Enterobacteriaceae family, Klebsiella does not require any particular growing conditions in order to thrive. Aerobic and facultatively anaerobic organisms. Their optimal conditions for growth include a temperature range of 35–37 °C and a pH of around 7.2. [21] When compared to other Enterobacteriaceae bacteria, Klebsiella often has a rounder and thicker shape. They usually manifest as rods that are straight and have ends that are either slightly pointed or rounded. They often appear alone, in pairs, or even in loose clusters. In vivo, diplobacillary forms are prevalent. [22] is a They do well in normal laboratory media and don't have any particular growth needs; however, they do best at temperatures between 35 and 37 °C with a pH of 7.2. Since they are facultative anaerobes, the majority of strains can get by on just citrate and glucose for carbon and ammonia for nitrogen. Although genetic serotyping has the potential to supersede the use of the genus's characteristic slime layer—a conspicuous capsule—for serologic identification, this method may no longer be necessary [23]. [24] It is common for Klebsiella bacteria to display two distinct antigens on their cell walls. The first of these is the O antigen, which is one of nine different types of lipopolysaccharide (LPS). Second, there are over eighty different kinds of the capsular polysaccharide known as K antigen. Both are essential for serogrouping and play a role in pathogenicity. These two antigenic determinants have served as the basis for multiple vaccinations. [25] The Normal flora of the human nose, mouth, and intestines include Klebsiella species; nevertheless, these same bacteria can also act as opportunistic human infections. As well as infecting humans, many other animals can contract Klebsiella bacteria, either naturally or as opportunistic diseases. Many different diseases can be caused by Klebsiella organisms. Some of the more common ones include pneumonia, UTIs, sepsis, meningitis, diarrhoea, peritonitis, and infections of the soft tissues. [27] The development of ankylosing spondylitis and other spondyloarthropathies has also been linked to Klebsiella species. Klebsiella pneumoniae and Klebsiella oxytoca are the most common types of Klebsiella infections in humans. Most infections include contamination of an invasive medical device, and they are more common in the very young, the very elderly, and people with serious underlying conditions like cancer. [29] Over the past four decades, researchers have utilised novel approaches to develop potent vaccines against Klebsiella and K. pneumoniae. But there isn't a Klebsiella vaccine available in the US just yet. Nosocomial respiratory tract infections and neonatal intensive care unit infections are most commonly caused by Klebsiella pneumoniae. Gram-negative bacteraemia and UTIs are most commonly caused by Klebsiella pneumoniae as well. Healthcare facilities continue to face the challenge of drug-resistant bacterial isolates, which prolong patient stays, increase healthcare costs, and pose a particular threat in high-impact medical settings like intensive care units. Multidrug efflux pumps are largely believed to be responsible for this antibiotic resistance.[30] One of the main reasons why hospital-acquired diseases can spread so easily is that Klebsiella pneumoniae can live on almost any surface in a healthcare facility, including carpets, sinks, flowers, and even the skin of patients and staff.[31]

***Klebsiella pneumoniae***

Klebsiella pneumoniae is a rod-shaped, facultative anaerobic, Gram-negative, non-motile bacteria that ferments lactose. It grows on MacConkey agar looking like a mucoid lactose fermentor. Even though it's a common part of the gut, skin, and mouth, aspirating it can harm the lungs of humans and animals, particularly the alveoli, leading to sputum that looks like jelly and is crimson, brownish, or yellow. It is the most important Klebsiella species in the clinical context of the Enterobacteriaceae family. Human clinical specimens have also shown the presence of K.

oxytoca and K. rhinoscleromatis. Nosocomial infections caused by Klebsiella species have emerged as a major health concern in the past few years.[32] It's a soil microbe that fixes nitrogen in anaerobic environments; around 30% of strains are capable of this. The nitrogen-fixation mechanism of K. pneumoniae, a free-living diazotroph, has been extensively investigated and is of interest to farmers since it has been shown to boost crop yields in agricultural settings. [33]

**Secondary Metabolites**

The total number of biological reactions that an organism performs is called its metabolism. In most cases, only very small molecules may be considered metabolites; they are both metabolic intermediates and products. While all viable cells include primary metabolites, the term "secondary" metabolites, first coined by A. Kossel in 1891, suggests that these metabolites are merely present and do not play a crucial role in an organism's survival. While secondary metabolites do originate in primary metabolism, they are not structurally essential to the living being. It differs from primary metabolites in that its absence does not instantly kill an organism, but it does make survival more difficult. Within a given phylogenetic group, it is found in species that are ecologically disadvantaged and is synthesised by them [34].

Many of the intermediates in primary metabolism are also intermediates in secondary metabolism, making it difficult to tell the two apart. Despite their status as a primary metabolite byproduct, amino acids are also unquestionably a secondary metabolite. While it's true that sterols are essential components of numerous cellular structures, this is not necessarily the case. According to [35], the fact that an intermediate is mosaic-shaped suggests that primary and secondary metabolism share a same metabolic pathway. Inactive primary metabolism can be diverted into the secondary metabolites, which operate as a buffer zone for excess carbon and nitrogen. Metabolic breakdown of secondary metabolites allows the stored carbon and nitrogen to return to primary metabolites upon need. Growth, tissue differentiation, and development within the cell or organism, as well as external forces, impact the activities of primary and secondary metabolism, which are dynamic and delicately balanced.

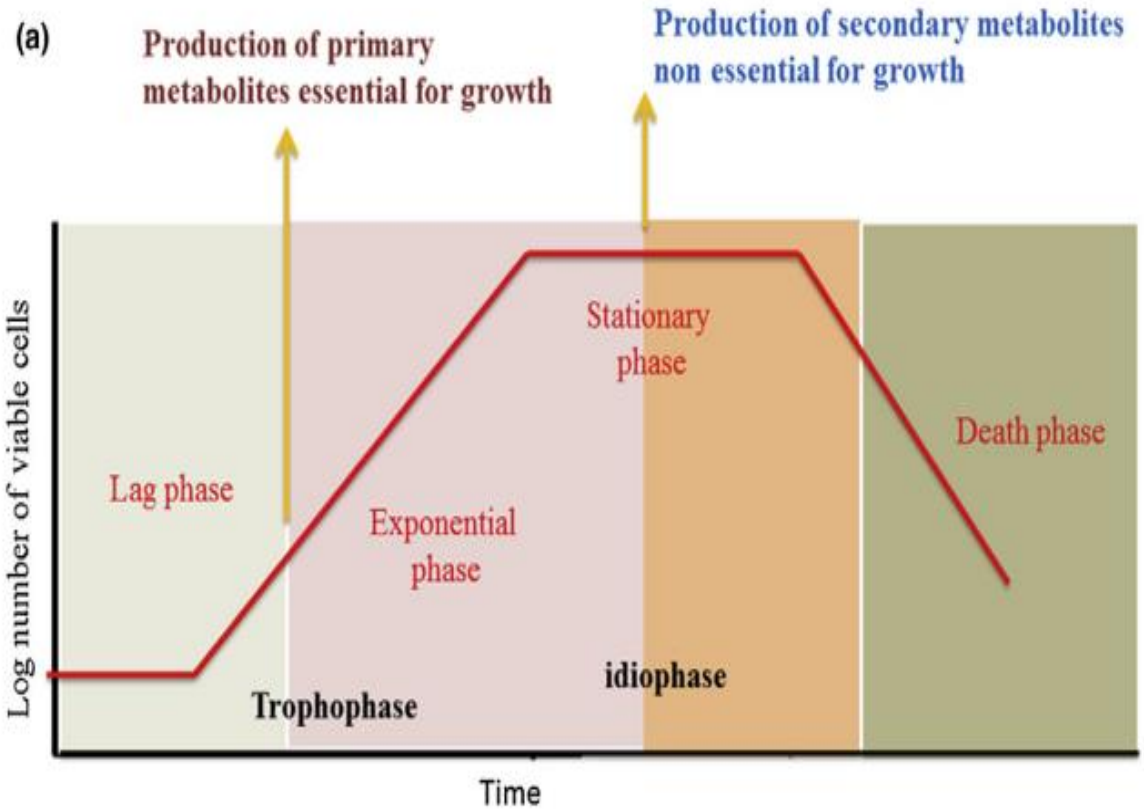
**Production of Secondary Metabolites of Bacteria**

The metabolic process is an ongoing biochemical activity that takes place in all living things, whether they are single-celled or multicellular. Catabolism and anabolism are the two main categories into which biochemical processes fall. "Metabolites" are the byproducts of these pathways that serve as building blocks for other metabolic processes. The various biological characteristics exhibited by metabolites have significant implications in the fields of agriculture, nutrition, and pharmaceuticals. Primary metabolites are defined as molecules that are involved in specific metabolic processes, while secondary metabolites are defined as those that are not. Amino acids, pyruvate, citric acid, and lactic acid are examples of basic metabolites that provide energy for cellular biochemistry and physiology. Secondary metabolites, on the other hand, aren't required for cell proliferation but instead help the organism stay alive in tough situations. Bacterial secondary metabolite synthesis is the subject of this chapter (Table 1). During their late and stationary phases of growth, the bacteria that produce secondary metabolites create these complex and bioactive compounds (Figure 1). When development conditions are restricted, environmental stress levels are high, or nutrients are depleted, the body responds by producing secondary metabolites. Bacteria, fungus, plants, and marine creatures are common sources of the secondary metabolites. Antibacterial agents, toxins, metal-transporting agents, sex hormones, pigments, anticancer agents, pesticides, immunomodulating agents, immunosuppressants, receptor agonists, and antagonists are all possible metabolites produced by these organisms. An enzyme or a set of enzymes catalyses a reaction in a secondary metabolic pathway. Figure 1(b) shows the metabolic routes that lead to the synthesis of

secondary metabolites, which are intermediate or end-products of primary metabolic pathways.

**Table 1. Biochemical and physiological properties of primary and secondary metabolites.**

| Primary metabolites | Secondary metabolites |
|---|---|
| • Small molecules<br>• Produces few intermediates or end-products<br>• End-products are building blocks for macromolecules.<br>• Essential for growth and cell viability<br>• Known physiological function<br>• Composed of simple chemical structure<br>• End-products are used for Coenzyme synthesis<br>• Production occurs at log phase<br>• Primary metabolites are used in food and feed industry<br>• Provides the energy for cellular activities | • Small molecules<br>• Produces array of molecules<br>• Synthesize new compounds<br>• Not vital for the cell growth<br>• Analysis of physiological function is difficult<br>• Products of complex unusual chemical structure<br>• End-products are used an antibacterial agent<br>• Production occurs at late and dormant phase<br>• Secondary metabolites are used in food, cosmetic, agricultural and farming industry<br>• Protects the organisms under various harsh environment |



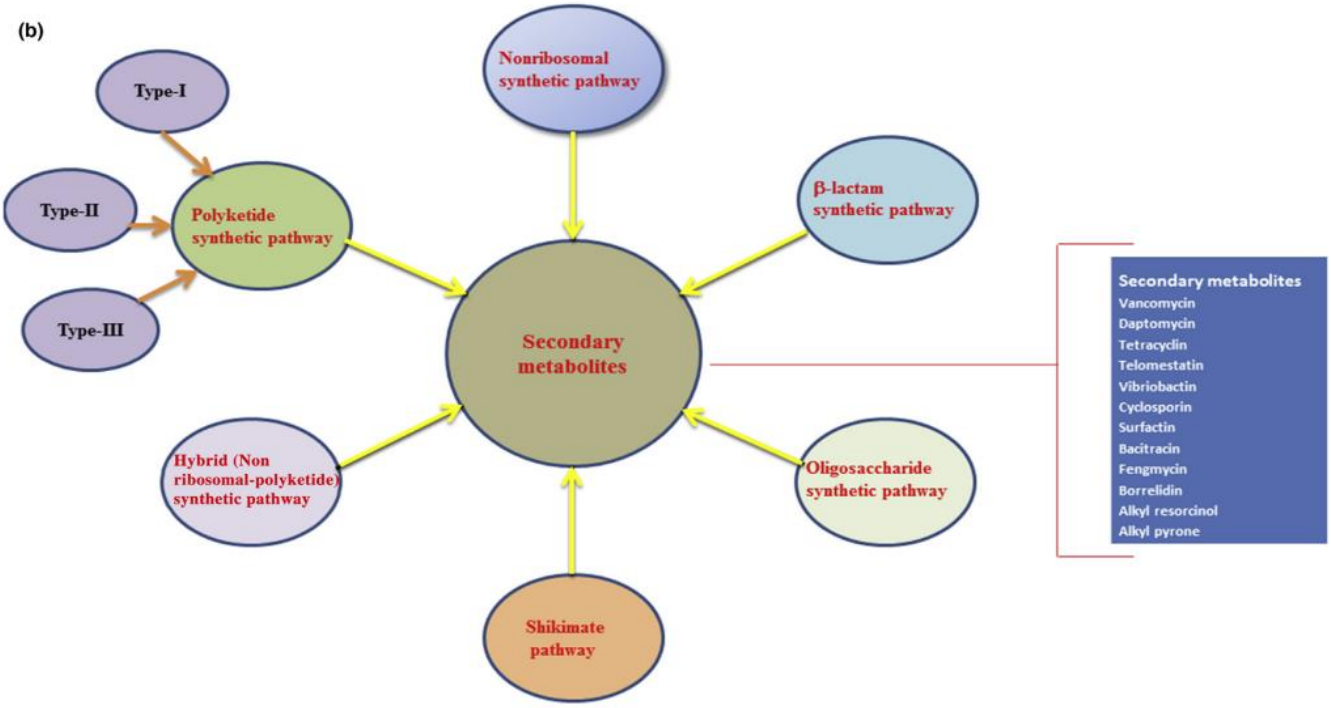



**Figure 1. (a) Various phases of bacterial growth and production of metabolites. The primary metabolites production generally occurs at the late lag phase and middle of exponential phase. The secondary metabolites production occurs at the end of the stationary phase and during the persistent phase. (b) Various pathways responsible for the assembly of secondary metabolites.**

## Secondary Metabolites of Microorganisms

Microbial secondary metabolites are low molecular mass products with unusual structures. The structurally diverse metabolites show a variety of biological activities like antimicrobial agents, inhibitors of enzymes and antitumors, immune-suppressives and antiparasitic agents [35,36], plant growth stimulators, herbicides, insecticides, antihelmintics, etc. They are produced during the late growth phase of the microorganisms. The secondary metabolite production is controlled by special regulatory mechanisms in microorganisms, as their production is generally repressed in logarithmic phase and depressed in stationary growth phases. The microbial secondary metabolites have distinctive molecular skeleton which is not found in the chemical libraries and about 40% of the microbial metabolites cannot be chemically synthesized.

## Features of microbial secondary metabolites

- The principle and process of natural fermentation product synthesis can be successfully scaled up and employed to maximize its application in the field of medicine, agriculture, food, and environment.
- The metabolite can serve as a starting material for deriving a product of interest, extended further through chemical or biological transformation.
- New analog or templates in which secondary metabolite serve as lead compounds will lead discovery and design of new drugs.

## Applications of Microbial Secondary Metabolites

### Antibiotics:

The discovery of penicillin initiated the researchers for the exploitation of microorganisms for secondary metabolite production, which revolutionized the field of microbiology [37]. With the advent of new screening and isolation techniques, a variety of β-lactam-containing molecules [38] and other types of antibiotics have been identified. About 6000 antibiotics have been described, 4000 from actinobacteria. In the

prokaryotic group, unicellular bacteria Bacillus and Pseudomonas species are the most recurrent antibiotic produc ers. Likewise in eukaryotes, fungi are dominant antibiotic producers next to plants. In the recent years, myxobacteria and cyanobacteria species have joined these distinguished organisms as productive species.

The pharmaceutical product, especially anti-infective derivatives comprise 62% antibacteri als, 13% sera, immunoglobulins, and vaccines, 12% anti-HIV antivirals, 7% antifungals, and 6% nonHIV antivirals. There are over 160 antibiotics. Streptomyces hygroscopicus with over 200 antibiotics, Streptomyces griseus with 40 antibiotics, and Bacillus subtilis with over 60 com pounds are the major contributors to the antibiotic market [39].

**Antitumor agents**

Natural product and its derivatives account for more than 60% of anticancer formula tions. Actinobacteria derived antineoplastic molecules currently in use are actinomycin D, anthracyclines (daunorubicin, doxorubicin, epirubicin, pirarubicin, and valrubicin), bleomy cin, mitosanes (mitomycin C), anthracenones (mithramycin, streptozotocin, and pentostatin), enediynes (calicheamicin), taxol, and epothilones [40]. Taxol is the nonactinobacterial molecule derived from plant Taxus brevifolia and endophytic fungi Taxomyces andreanae and Nodulisporium sylviforme. It interferes with microtubule break down, an essential event leading to cell division, thereby inhibiting rapidly dividing cancer cells. It is effective against breast and advanced form Kaposi's sarcoma. It is also found to exhibit antifungal activity against Pythium, Phytophthora, and Aphanomyces.[41]

**Pharmacological and nutraceutical agents**

One huge success was the discovery of the fungal statins, including compactin, lovastatin, pravastatin, and others which act as cholesterol-lowering agents. Lovastatin is produced by A. terreus. Of great importance in human medicine are the immunosuppressants such as cyclo sporin A, sirolimus (rapamycin), tacrolimus, and mycophenolate mofetil. They are used for heart, liver, and kidney transplants and were responsible for the establishment of the organ transplant field. Cyclosporin A is made by the fungus Tolypocladium niveum. Mycophenolate mofetil is a semisynthetic product of the oldest known antibiotic, mycophenolic acid, and is also made by a fungus. Sirolimus and tacrolimus are products of streptomycetes [42].

Metabolites of probiotic bacteria are considered as a remedy for controlling weight gain, pre venting obesity, increasing satiety, prolonging satiation, reducing food intake, reducing fat deposition, improving energy metabolism, treating and enhancing insulin sensitivity, and treating obesity. Firmicutes and Bacteroidetes are the dominant beneficial bacteria present in the normal human gastrointestinal tract, and the latter was reported in lower numbers in constipation-predominant irritable bowel syndrome patients [43].

Carotenoids of microbial origin are used as food colorant, fish feeds, nutraceuticals, cosmetics, and antioxidants. Food colorant widely used is carotene derived from Blakeslea trispora, Dunaliella salina and lycopene from B. trispora and Streptomyces chrestomyceticus, subsp. rubescens. Astaxanthin produced from Xanthophyllomyces dendrorhous is an approved fish feed. Astaxanthin, lutein, β-carotene, zeaxanthin, and canthaxanthin are used as nutraceuticals due to their excellent antioxidant property. Docosahexaenoic acid (DHA) used in infant formula as nutritional supplements is derived from microalgae Schizochytrium spp. [44].

**Enzymes and enzyme inhibitors**

Enzymes produced from microorganism have annual sales of US $ 2.3 billion enzymes that find application in detergents (34%), foods (27%), agriculture and feeds (16%), textiles (10%), and leather, chemicals, and pulp and paper (10%). The protease subtilisin used in detergents has an annual sale of $ 200 million. The other major enzymes include glucose isomerase (100,000 tons) and penicillin amidase (60,000 tons). Nitrilase (30,000 tons) and phytase are amounting for $135 million worth of production.

Streptomyces glucose isomerase is used to isomerize D-glucose to D-fructose, to make 15 million tons per year of high fructose corn syrup, valued at $1 billion [45].

The most important enzyme inhibitors are clavulanic acid, synthesized by Streptomyces cla vuligerus, the inhibitor of β-lactamases. Some of the common targets for other inhibitors are glucosidases, amylases, lipases, proteases, and xanthine oxidase. Amylase inhibitors prevent absorption of dietary starches into the body, and hence can be used for weight loss.

**Agricultural and animal health products**

Secondary metabolites find wide applications in the field of agriculture and animal health: kasugamycin and polyoxins are used as biopesticides; Bacillus thuringiensis crystals, nikkomy cin, and spinosyns are used as bioinsecticides; bioherbicides (bialaphos) find application as bioherbicides; ivermectin and doramectin as antihelmintics and endectocides against worms, lice, ticks, and mites; ruminant growth promoters in the form of coccidiostats; plant hormones like gibberellins as growth regulators are the most common application [46].

**Production of secondary metabolites from microorganisms**

1. Liquid fermentation Batch or fed-batch culture in submerged fermentation is employed for production of secondary metabolites. Inoculum is developed after careful strain improvement of producing organism. Initially, shake flasks culture is employed, and the culture which are in active growth phase are transferred to a small fermenter and later into a larger fermenter with production medium. Several parameters, like medium composition, pH, temperature, and agitation and aeration rate, are controlled [47,48]. An inducer such as methionine is added to cephalosporin fermentations, phosphate is restricted in chlortetracycline fermentation, and glucose is avoided in penicillin or erythromycin fermentation.
2. Solid-state fermentation Solid-state fermentation, defined as a microbial culture that develops on the surface and at the interior of a solid matrix and in the absence of free water, holds an important potential for the production of secondary metabolites [49,50]. Two types of SSF can be distinguished, depending on the nature of solid phase used: (a) solid culture of one support-substrate phase solid phase and (b) solid culture of two substrate-support phase solid phase [51]. The advantages of solid state fermentation in relation with submerged fermentation include: energy requirements of the process are relatively low, since oxygen is transferred directly to the microorganism. Secondary metabolites are often produced in much higher yields, often in shorter times, and often sterile conditions are not required.

**Materials and Methods**

**Growth conditions and determination of metabolites**

*Klebsiella pneumoniae* strain was isolated from bronchitis patients and obtained from Maternity and children hospital. Subcultures were obtained on the Nutrient Agar for 48 hrs. at 22°C. The mixture was incubated at 4ºC for 10 min and then shook for 10 min at 130 rpm. Metabolites was separated from the liquid culture and evaporated to dryness with a rotary evaporator at 45ºC.

The residue was dissolved in 1 ml methanol, filtered through a 0.2 µm syringe filter, and stored at 4ºC for 24 h before being used for GC-MS. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library as well as on comparison of their retention indices either with those of authentic compounds or with literature values.

**Spectral analysis of bioactive natural chemical compounds of *Klebsiella pneumoniae* using (GC/MS)**

Analysis was conducted using GC-MS (Agilent 789 A) equipped with a DB-5MS column (30

m×0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as for the previous analysis. Helium was used as the carrier gas at the rate of 1.0 mL/min. Effluent of the GC column was introduced directly into the source of the MS via a transfer line (250oC). Ionization voltage was 70 eV and ion source temperature was 230oC. Scan range was 41- 450 amu. The components were identified by comparing their retention times to those of authentic samples of WILEY MASS SPECTRAL DATA BASE Library.

**Determination of antibacterial activity**

Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions (Metabolites Produced by *Klebsiella pneumonia*) were delivered into the wells. The test pathogens (*Streptococcus pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis* and *Staphylococcus epidermidis*) were swabbed in Muller Hinton agar plates. 90µl of fungal extracts was loaded on the bored wells. The wells were bored in 0.5cm in diameter. The plates were incubated at 37C° for 24 hrs and examined. Methanol was used as solvent control.

**Data analysis**

All the measurements were replicated three times for each assay and the results are presented as mean ± SD and mean ± SE. IBM SPSS 20 version statistical software package was used for statistical analysis of percentage inhibition and disease incidence and disease severity in each case.

**Results and Discussion**

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *Klebsiella pneumoniae*, shown in **Figure 1-40.** Peaks were determined to be Tricyclo[4.3.1.1(3.8)]undecan-1-amine, 3-Methoxybenzaldehyde semicarbazone, carboxaldehyde , 1-methyl-,oxime ,(Z)-(+), 1,5,5-Trimethyl-6-methylene-cyclohexene, 4-(2,5-Dihydro-3-methoxyphenyl)butylamine, Paromomycin , 9-Borabicyclo[3.31]nonane , 9-mercapto-, Benzenemethanol , 2-(2-aminopropoxy)-3-methyl, Acetamide , N-(6-acetylaminobenzothiazol-2-yl)-2-(adamantan, rin-6-carboxylic acid , 4-(2,5-Dihydro-3-methoxyphenyl)butylamine, N-(2,5-Dicyano-3,4-dihydro-2H-pyrrol-2-yl)-acetamide, 3,10-Dioxatricyclo [4.3.1.0(2,4)]dec-7-ene, 3-Cyclohex-3-enyl-propionic acid, Eicosanoic acid ,phenylmethyl ester, 3,7-Diazabicyclo[3.3.1]nonane , 9,9-dimethyl-, Dithiocarbamate , S-methyl-,N-(2-methyl-3-oxobutyl)-, dl-Homocysteine, 2-(2-Furyl)pyridine, 1,7-Dioxa-10-thia-4,13-diazacyclopentadeca-5,9,12-trione, 5,7-Dodecadiyn-1,12-diol, 1-(β-d-Arabinofuranosyl)-4-O-difluoromethyluracil, Uric acid, Pyrrolo[1.2-a]pyrazine-1,4-dione , hexahydro-,12-Methyl-oxa-cyclododecan-2-one, Phthalic acid , butyl undecyl ester, 9,12,15-Octadecatrienoic acid , 2,3-bis(acetyloxy)propyl ester, 1,2,4-Trioxolane-2-octanoic acid 5-octyl-, methyl ester, 12-Dimethylamino-10-oxododecanoic acid , Octahydrochromen-2-one, L-Aspartic acid , N-glycyl-,2H-Oxecin-2-one , 3,4,7,8,91,10-hexahydro-4-hydroxy-10-meth , Thiazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione , 2-amino-4-(2-ph, Dec-9-en-6-oxo-1-ylamide, 3,6,12-Trimethyl-1,4,7,10,13,16-hexaaza-cyclooctadecane, 2-lodohiistidine, 2,5-Piperazinedione ,3,6-bis(2-methylpropyl)-, 9-Octadecenamide , (Z)-, 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetra.

**Antibacterial activity**

Clinical pathogens selected for antibacterial activity namely, *Streptococcus pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Staphylococcus epidermidis*, It were 4.09±0.013, 2.99±0.300, 4.37±0.200, 3.22±0.210, and 4.00±0.203 respectively for Bacterial products (Metabolites Produced by *Klebsiella pneumoniae*), while recorded 1.08±0.200, 0.97±0.116, 2.08±0.233, 3.04±0.261, 0.98±0.166 respectively for Bacterial products Streptomycin antibiotics, and recorded 1.02±0.180, 1.00±0.190, 2.08±0.236, 1.00±0.100, and 1.82±0.200 respectively for Kanamycin antibiotics.

**Table 1. Bioactive chemical compounds identified in methanolic extract of *Klebsiella pneumoniae*.**

| / Bacterial products Antibiotics | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | Bacteria | | | | |
| | ***Streptococcus pneumonia*** | ***Escherichia coli*** | ***Staphylococcus aureus*** | ***Proteus mirabilis*** | ***Staphylococcus epidermidis*** |
| **Bacterial products** | 4.09±0.013 | 2.99±0.300 | 4.37±0.200 | 3.22±0.210 | 4.00±0.203 |
| **Streptomycin** | 1.08±0.200 | 0.97±0.116 | 2.08±0.233 | 3.04±0.261 | 0.98±0.166 |
| **Kanamycin** | 1.02±0.180 | 1.00±0.190 | 2.08±0.236 | 1.00±0.100 | 1.82±0.200 |

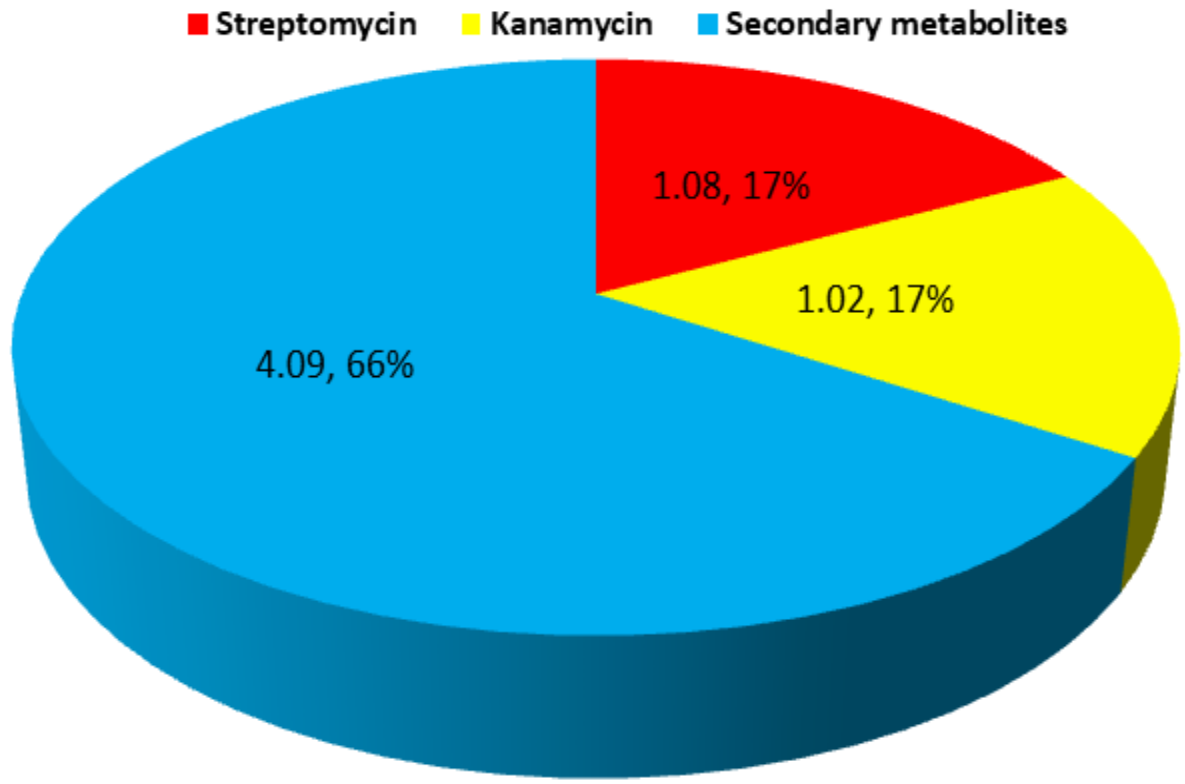



**Figure 41. Metabolite products, Streptomycin and Kanamycin as anti-Bacterial activity against *Streptococcus pneumonia***

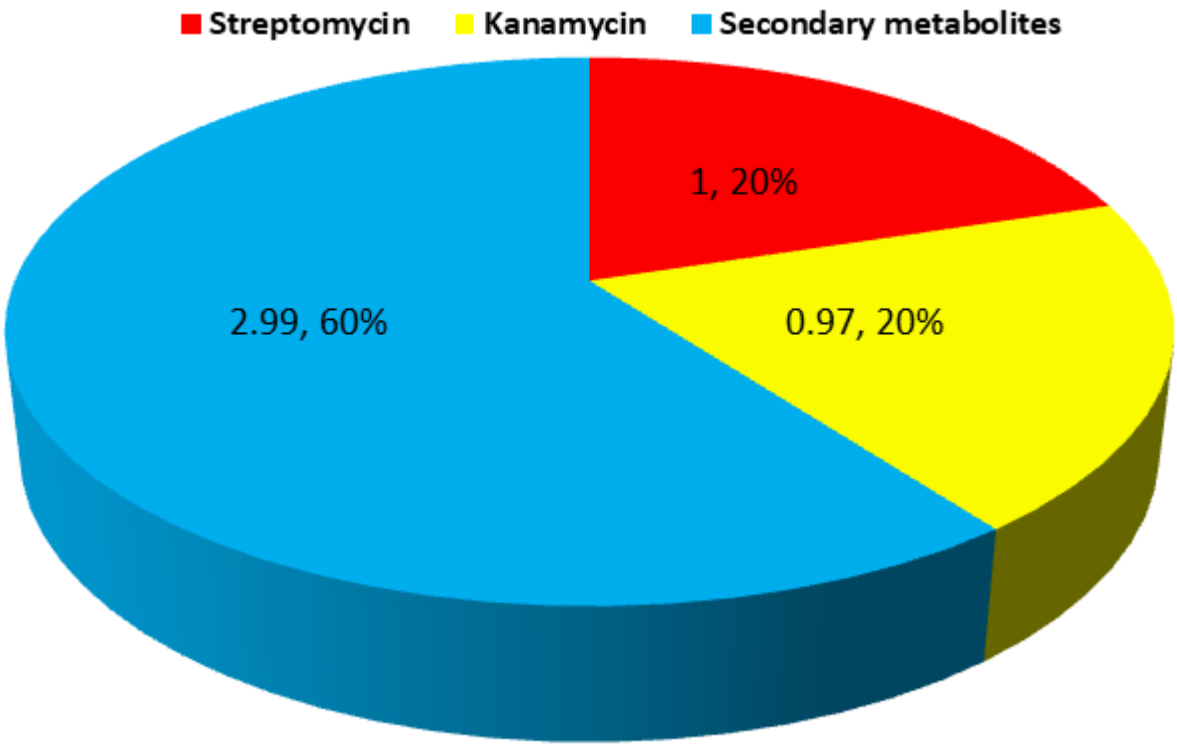



**Figure 42. Metabolite products, Streptomycin and Kanamycin as anti-Bacterial activity against *Escherichia coli***

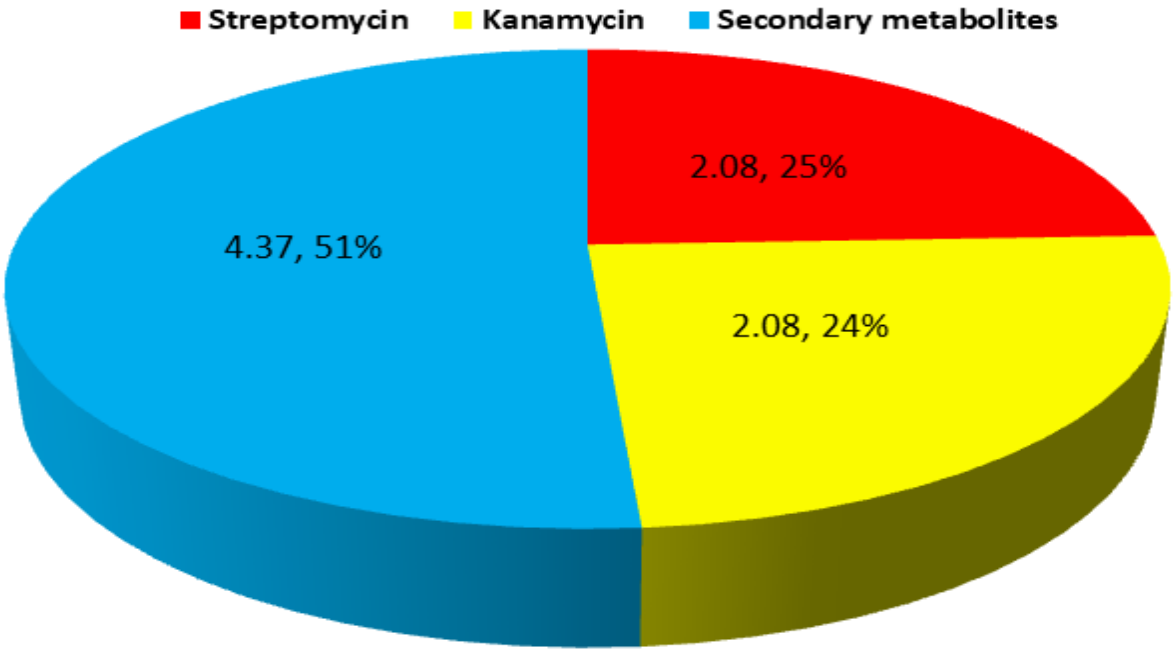



**Figure 43. Metabolite products, Streptomycin and Kanamycin as anti-Bacterial activity against *Staphylococcus aureus***

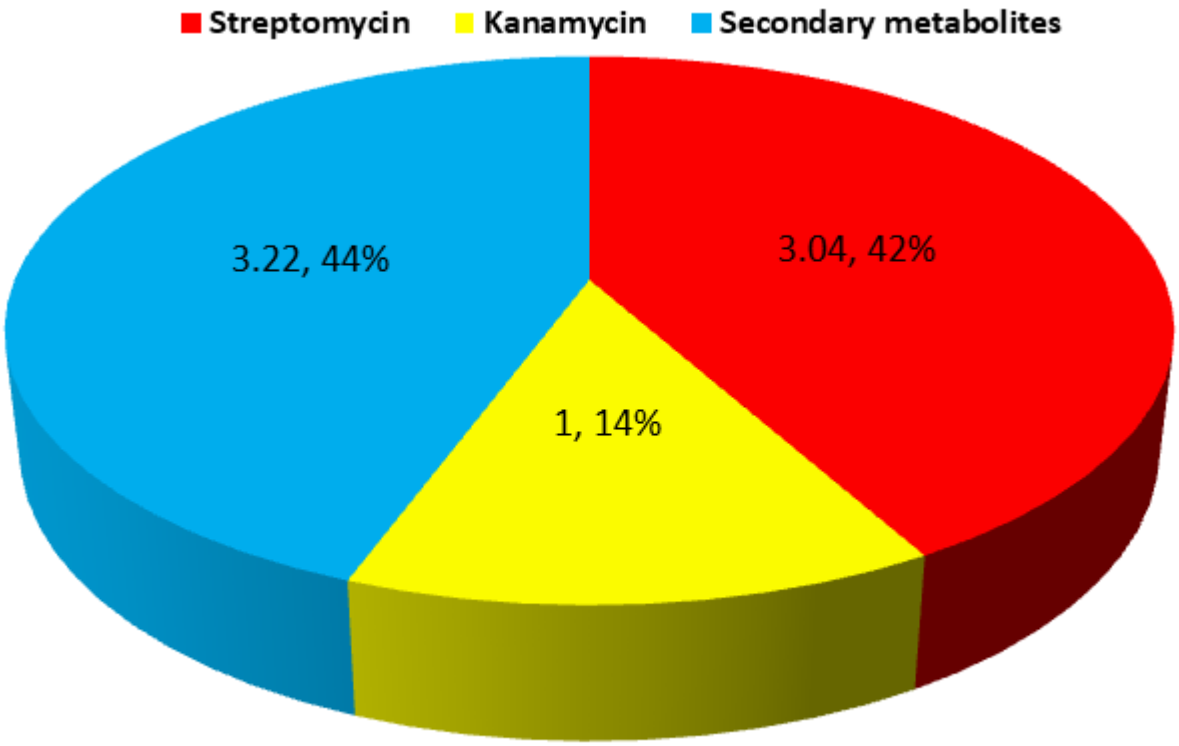



**Figure 44. Metabolite products, Streptomycin and Kanamycin as anti-Bacterial activity against *Proteus mirabilis***

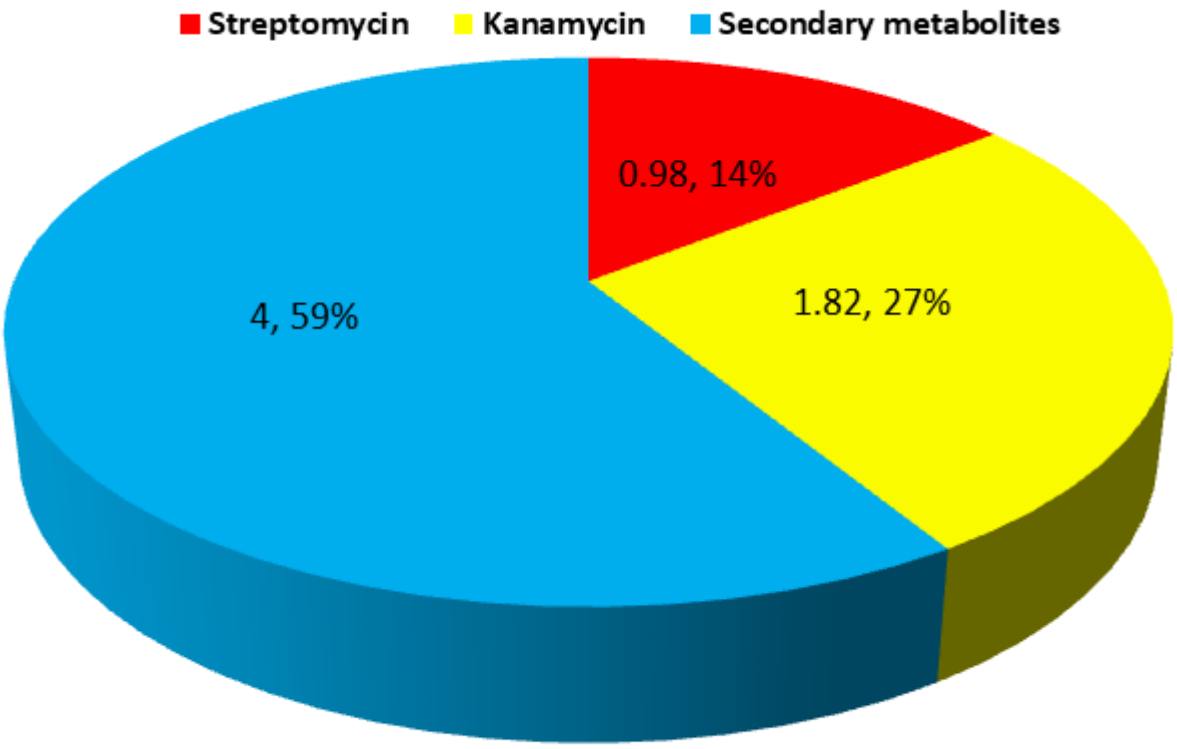



**Figure 45. Metabolite products, Streptomycin and Kanamycin as anti-Bacterial activity against *Staphylococcus epidermidis***

Maximum zone formation against *Staphylococcus aureus* (4.37±0.200) mm, Table 1. Klebsiella pneumoniae produce many important secondary metabolites with high biological activities. Based

on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *Klebsiella pneumonia* can be useful.

As a general rule, *Klebsiella* infections are seen mostly in people with a weakened immune system. Most often, illness affects middle-aged and older men with debilitating diseases. This patient population is believed to have impaired respiratory host defenses, including persons with diabetes, alcoholism, malignancy, liver disease, chronic obstructive pulmonary diseases, glucocorticoid therapy, renal failure, and certain occupational exposures (such as papermill workers). Many of these infections are obtained when a person is in the hospital for some other reason (a nosocomial infection). Feces are the most significant source of patient infection, followed by contact with contaminated instruments.

## Conclusion

Microorganisms have the capability to produce a number of antibiotics and other pharmaceutically important drugs to treat bacterial and fungal infections, cancer, and heart-related diseases. Bacterial species exhibit a complex life cycle with a physiological and biochemical adaptability, with the capability to synthesize a great variety of secondary metabolites with complex structures using different metabolic pathways. Understanding the secondary metabolite biosynthesis and pathways will lead to progress in combinatorial biosynthesis in the pharmaceutical and biotechnology industries. Thirty nine bioactive chemical constituents have been identified from methanolic extract of the *Klebsiella pneumoniae* by gas chromatogram mass spectrometry (GC-MS). In vitro antifungal and antibacterial evaluation of secondary metabolite products of *Klebsiella pneumoniae* forms a primary platform for further phytochemical and pharmacological investigation for the development of new potential antimicrobial compounds.

## Acknowlgements

I extend my thanks and appreciation to everyone who contributed with us for providing me the opportunity to work on this project. I am thankful to you for helping me through the various analysis stages, and for providing helpful criticism and feedback throughout the writing process.

## References

1. Ryan KJ, Ray CG. Sherris Medical Microbiology (4th ed.). McGraw Hill. *ISBN*. 2004; 8385-8529-9.
2. Postgate J. Nitrogen Fixation (3rd ed). *Cambridge University Press. ISBN*. 1998; 978-0-521-64047-3.
3. Riggs PJ, Chelius MK, Iniguez AL, Kaeppler SM, Triplett EW. "Enhanced maize productivity by inoculation with diazotrophic bacteria". *Australian Journal of Plant Physiology*. 2001; 29 (8): 829–836.
4. Podschun R, Ullmann U . "Klebsiella spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors". *Clinical Microbiology Reviews*. 1998; 11 (4): 589–603. PMC 88898.
5. Rashid T, Ebringer A . "Ankylosing spondylitis is linked to Klebsiella--the evidence". *Clinical Rheumatology*. 2007; 26 (3): 858–864.
6. Groopman J (2008). "Superbugs". The New Yorker. Retrieved 2013-07-07. The new generation of resistant infections is almost impossible to treat.
7. Hudson, Corey; Bent, Zachary; Meagher, Robert; Williams, Kelly ."Resistance Determinants and Mobile Genetic Elements of an NDM-1-Encoding Klebsiella pneumoniae Strain". *PLOS ONE*. 2014; 9: e99209.
8. Nathisuwan S, Burgess DS, Lewis JS. Extended-Spectrum β-Lactamases:

Epidemiology, Detection, and Treatment". *Pharmacothe*r. 2001; 21 (8): 920–928.

9. Limbago BM, Rasheed, JK, Anderson KF, Zhu W. "IMP-Producing Carbapenem-Resistant Klebsiella pneumoniae in the United States". *Journal of Clinical Microbiology*. 2011; 49 (12): 4239–4245.
10. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez, A. "Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae". *Antimicrobial Agents and Chemotherapy*. 2001; 45 (4): 1151–1161.
11. Lledo W, Hernandez M, Lopez E, Molinari OL."Guidance for Control of Infections with Carbapenem-Resistant or Carbapenemase-Producing Enterobacteriaceae in Acute Care Facilities". *Morbidity and Mortality Weekly Report. CDC*. 2009; 58 (10): 256–260.
12. Schwaber MJ, Lev B, Israeli A, Solter E. "Containment of a country-wide outbreak of carbapenem-resistant Klebsiella pneumoniae in Israeli hospitals via a nationally implemented intervention". *Clinical Infectious Diseases*. 2011; 52 (7): 848–855.
13. Bogovazova GG, Voroshilova NN, Bondarenko VM . "The efficacy of Klebsiella pneumoniae bacteriophage in the therapy of experimental Klebsiella infection". Zhurnal mikrobiologii, epidemiologii, i immunobiologii (in Russian). Russia: *Moskva* 1991;(4): 5–8.
14. Chanishvil N, ed. A Literature Review of the Practical Application of Bacteriophage Research. Hauppauge, NY: *Nova Science*. 2012;ISBN 978-1-62100-851-4.
15. Kadhim MJ, Sosa AA, Hameed IH. Evaluation of anti-bacterial activity and bioactive chemical analysis of *Ocimum basilicum* using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(6): 127-146.
16. Mohammed GJ, Kadhim MJ, Hussein HM. Characterization of bioactive chemical compounds from *Aspergillus terreus* and evaluation of antibacterial and antifungal activity. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(6): 889-905.
17. Hameed IH, Altameme HJ, Idan SA. Artemisia annua: Biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2016; 7(2): 1843-1868
18. Hussein AO, Mohammed GJ, Hadi MY, Hameed IH. Phytochemical screening of methanolic dried galls extract of *Quercus infectoria* using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR). *Journal of Pharmacognosy and Phytotherapy*. 2016; 8(3): 49-59.
19. Sosa AA, Bagi SH, Hameed IH. Analysis of bioactive chemical compounds of *Euphorbia lathyrus* using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(5): 109-126.
20. Altameme HJ, Hadi MY, Hameed IH. Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. J*ournal of Pharmacognosy and Phytotherapy*. 2015; 7(10): 238-252.
21. Mohammed GJ, Omran AM, Hussein HM. Antibacterial and Phytochemical Analysis of *Piper nigrum* using Gas Chromatography-Mass Spectrum and Fourier-Transform Infrared Spectroscopy.

*International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(6): 977-996.

22. Hamza LF, Kamal SA, Hameed IH. Determination of metabolites products by *Penicillium expansum* and evaluating antimicobial activity. *Journal of Pharmacognosy and Phytotherapy*. 2015; 7(9): 194-220.
23. Jasim H, Hussein AO, Hameed IH, Kareem MA. Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*. 2015; 7(4): 56-72.
24. Hadi MY, Mohammed GJ, Hameed IH. Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*. 2016; 8(2): 8-24.
25. Hameed IH, Ibraheam IA, Kadhim HJ. Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of *Rosmarinus oficinalis* leaves. J*ournal of Pharmacognosy and Phytotherapy*. 2015; 7 (6): 90-106.
26. Shareef HK, Muhammed HJ, Hussein HM, Hameed IH. Antibacterial effect of ginger (*Zingiber officinale*) roscoe and bioactive chemical analysis using gas chromatography mass spectrum. *Oriental Journal of Chemistry*. 2016; 32(2): 20-40.
27. Al-Jassaci MJ, Mohammed GJ, Hameed IH. Secondary Metabolites Analysis of *Saccharomyces cerievisiae* and Evaluation of Antibacterial Activity. *International Journal of Pharmaceutical and Clinical Research*. 2016; 8(5): 304-315.
28. Mohammed GJ, Al-Jassani MJ, Hameed IH. Anti-bacterial, Antifungal Activity and Chemical analysis of *Punica grantanum* (Pomegranate peel) using GC-MS and FTIR spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(3): 480-494.
29. Al-Marzoqi AH, Hadi MY, Hameed IH. Determination of metabolites products by *Cassia angustifolia* and evaluate antimicobial activity. *Journal of Pharmacognosy and Phytotherapy*. 2016; 8(2): 25-48.
30. Altameme HJ, Hameed IH, Abu-Serag NA. Analysis of bioactive phytochemical compounds of two medicinal plants, *Equisetum arvense* and *Alchemila valgaris* seed using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *Malays. Appl. Biol.* 2015; 44(4): 47–58.
31. Hameed IH, Hamza LF, Kamal SA. Analysis of bioactive chemical compounds of *Aspergillus niger* by using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *Journal of Pharmacognosy and Phytotherapy*. 2015;7(8): 132-163.
32. Hameed IH, Hussein HJ, Kareem MA, Hamad NS. Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*. 2015; 7 (7): 107-125.
33. Hussein HM, Hameed IH, Ibraheem OA. Antimicrobial Activity and spectral chemical analysis of methanolic leaves extract of *Adiantum Capillus-Veneris* using GC-MS and FT-IR spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(3): 369-385.
34. Hussein HJ, Hadi MY, Hameed IH. Study of chemical composition of *Foeniculum vulgare* using Fourier transform infrared spectrophotometer and gas chromatography - mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*. 2016; 8(3): 60-89.

35. Kadhim MJ, Mohammed GJ, Hameed IH. In *vitro* antibacterial, antifungal and phytochemical analysis of methanolic fruit extract of *Cassia fistula*. *Oriental Journal of Chemistry*. 2016; 32(2): 10-30.
36. Altameme HJ, Hameed IH, Idan SA, Hadi MY. Biochemical analysis of *Origanum vulgare* seeds by fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*. 2015; 7(9): 221-237.
37. Hussein HM. Determination of phytochemical composition and ten elements content (CD, CA, CR, CO, FE, PB, MG, MN, NI AND ZN) of *CARDARIA DRABA* by GC-MS, FT-IR and AAS technique. *Int. J Pharm Bio Sci*. 2016; 7(3): (B) 1009 –1017.
38. Hussein HM. Analysis of trace heavy metals and volatile chemical compounds of *Lepidium sativum* using atomic absorption spectroscopy, gas chromatography-mass spectrometric and fourier-transform infrared spectroscopy. Resea*rch Journal of Pharmaceutical, Biological and Chemical Sciences.* 2016; 7(4): 2529 – 2555.
39. Hameed IH. A new polymorphic positions discovered in mitochondrial DNA hypervariable region HVIII from central and north-central of Iraq. *Mitochondrial DNA*. 2016; 27(5): 3250-4.
40. Jaddoa HH, Hameed IH, Mohammed GJ. Analysis of volatile metabolites released by *Staphylococcus aureus* using gas chromatography-Mass spectrometry and determination of its antifungal activity. *Orient J Chem*. 2016; 32(4).
41. Hameed IH, Salman HD, Mohammed GJ. Evaluation of antifungal and antibacterial activity and analysis of bioactive phytochemical compounds of *Cinnamomum zeylanicum* (Cinnamon bark) using gas chromatography-mass spectrometry. *Orient J Chem*. 2016; 32(4).
42. Hameed IH, Jebor MA, Ommer AJ, Abdulzahra AI. Haplotype data of mitochondrial DNA coding region encompassing nucleotide positions 11,719–12,184 and evaluate the importance of these positions for forensic genetic purposes in Iraq. *Mitochondrial DNA*. 2016; 27(2): 1324-1327.
43. Kadhim MJ, Mohammed GJ, Hussein HM. Analysis of bioactive metabolites from *Candida albicans* using (GC-MS) and evaluation of antibacterial activity. *International Journal of Pharmaceutical and Clinical Research*. 2016; 8(7): 655-670.
44. Mohammad A, Imad H. Autosomal STR: From locus information to next generation sequencing technology. *Research Journal of Biotechnology*. 2013.
45. Hameed, I.H., Abdulzahra, A.I., Jebor, M.A., Kqueen, C.Y., Ommer, A.J. Haplotypes and variable position detection in the mitochondrial DNA coding region encompassing nucleotide positions 10,716-11,184. *Mitochondrial DNA*. 2015.
46. Ubaid JM, Hussein HM, Hameed IH. Analysis of bioactive compounds of *Tribolium castaneum* and evaluation of anti-bacterial activity. *International Journal of Pharmaceutical and Clinical Research*. 2016; 8(7): 655-670.
47. Altaee N, Kadhim MJ, Hameed IH. Detection of volatile compounds produced by *Pseudomonas aeruginosa* isolated from UTI patients by gas chromatography-mass spectrometry. *International Journal of Current Pharmaceutical Review and Research*. 2017; 7(6).
48. Altaee N, Kadhim MJ, Hameed IH. Characterization of metabolites produced by *E. coli* and analysis of its chemical compounds using GC-MS. *International Journal of Current Pharmaceutical Review and Research*. 2017; 7(6).
49. Hussein JH, Ubaid JM, Hameed IH. Gas chromatography – mass spectrum analysis of volatile components of methanolic

leaves extract of *Cordia myxa*. *International Journal of Current Pharmaceutical Review and Research*. 2017; 7(6).

50. Kadhim MJ, Kaizal AF, Hameed IH. Medicinal plants used for treatment of rheumatoid arthritis: A review. *International Journal of Pharmaceutical and Clinical Research*. 2017; 8(11).
51. Hameed, I.H., Al-Rubaye A.F. and Kadhim, M.J. Antimicrobial Activity of Medicinal Plants and Urinary Tract Infections. *International Journal of Pharmaceutical and Clinical Research*. 2017; 8(11).
52. Kadhim WA, Kadhim, M.J., Hameed, I.H. Antibacterial Activity of Several Plant Extracts Against *Proteus Species*. *International Journal of Pharmaceutical and Clinical Research*. 2017; 8(11).
53. Ubaid JM, Kadhim MJ, Hameed IH. Study of bioactive methanolic extract of *Camponotus fellah* using Gas chromatography – mass spectrum. *International Journal of Current Pharmaceutical Review and Research*. 2017; 7(6).